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REDUCED GLUTATHIONE CONTENT OF RETICULOCYTES AND  
NORMOCYTES

[Following is the translation of an article by F. Belfiore, S. Ferlito, and J. Meldolesi in the Italian-language periodical Bulletino della Societa Italiana di Biologia Sperimentale (Bulletin of the Italian Experimental Biology Society), Naples, Vol 39, No 23, 1963, pages 1580-1582.]

From the Institute of Pathological Medicine of Catania University.  
Catania section -- 26 July 1963 session.

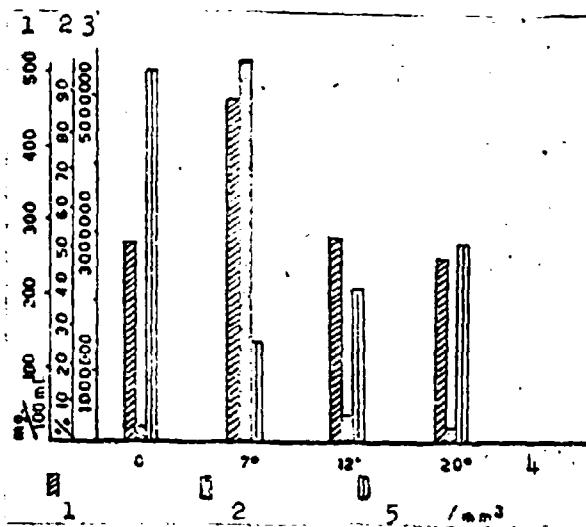
In earlier research on the enzyme metabolism inheritance of the young, morphologically reticulated erythrocyte, we discovered a considerable increase in the malic dehydrogenase (1), glutamic-oxalacetic and glutamic-pyruvic transaminase and arginase activity (2), and in the aldolase, glucose-6-phosphate dehydrogenase, phosphohexoseisomerase, and lactic dehydrogenase (3), pyruvate kinase, phosphogluconate dehydrogenase (4), isocitric dehydrogenase (5) activity and in the ATP content (6); on the other hand, we were unable to observe any significant change in the acid phosphatase activity (7) and in the ATP-ase activity (8).

In this article we are going to describe the behavior of reduced glutathione (GSH) which appears particularly interesting to us not only because of the great importance of glutathione in the metabolism of the erythrocyte but also with a view to the correct interpretation of the changes in glutathionemia in those syndromes which are characterized by intense reticulocytosis. On the basis of earlier observations (8, 9) we can say that the content of reduced glutathione in the young human red blood corpuscles -- separated according to the method of fractionated ultracentrifugation -- does not reveal any significant changes.

Materials and methods. The investigation was conducted on two groups of 10 rabbits of both sexes with an average weight of 1.5 kg. The animals in one group were treated with a daily injection of 10 mg of acetylphenyl-

hydrazine per kg of weight for a period of 5 days; the animals in the second group were bled daily for an amount of about 25 ml for a period of 5 days. Before the start of treatment and on the 7th, 12th, and 20th days we took blood samples by means of cardiac puncture using a heparinized syringe. We made a count of the erythrocytes and reticulocytes in these samples, using brilliant-blue cresyl staining; finally, using the washed red blood corpuscles, centrifuged at 3,000 rpm for a period of 10 minutes and then lysed with distilled water, we determined the reduced glutathione. This determination was made using the method of Grunert and Philipps as modified by Beutler (10); the GSH was expressed in mg per 100 ml of red blood corpuscles.

**Results.** The results obtained in the group of animals treated with acetylphenylhydrazine (see figure below) indicate that, before the start of the experiment (and, therefore, under normal conditions), the average number of red blood corpuscles per cu mm was 5,270,000, the percentage of reticulocytes was 2.7, and the glutathione content was 272 mg per 100 ml of red blood corpuscles. On the 7th day of the experiment -- that is, at the moment of maximum anemization and most intensive reticulocytosis (1,420,000 red blood corpuscles per cu mm and 95% reticulocytes), the reduced glutathione was 462 mg per 100 ml; compared to the basic values, this was an increase of 69%, which was statistically significant ( $p < 0.01$ ); on the 13th and 20th days, the progressive normalization of hematological conditions (2,510,000 and 3,593,000 red blood corpuscles per cu mm; reticulocyte percentages 7 and 2.5, respectively), was paralleled by a prompt normalization in the content of reduced glutathione which was 274 and 261 mg per 100 ml of red blood corpuscles.



Reduced glutathione content of rabbit erythrocytes during experimental hemolytic anemia. Legend: (1) GSH; (2) reticulocytes; (3) erythrocytes; (4) days of treatment; (5) red blood corpuscles.

The second group of animals -- in which reticulocytosis had been produced by means of repeated bleeding -- we used as control in order to determine whether the changes in the glutathione content, as revealed in the first group, were influenced by the toxic action of the hemolyzing substance. Starting from the same basic conditions, we noted, on the 7th day, that a red blood count of 7,593,000 per cu mm and a reticulocytosis of 41%, was paralleled by a glutathione content of 332 mg per 100 ml of red blood corpuscles; compared to the initial conditions, we had a 21.7% increase which was statistically significant ( $p < 0.02$ ). On the 12th day there was still a certain degree of anemia (2,900,000 red blood corpuscles per cu mm) and the reticulocytosis had dropped to 15%; at that time the glutathione had returned to normal limits (271 mg per 100 ml of red blood corpuscles). Considering the difference in reticulocytosis, it is obvious that the results obtained in the two groups of animals are sufficiently in agreement.

A number of nonreticulated elements is also present in the phase of maximum reticulocytosis; we can therefore say that the glutathione content of the reticulocyte was greater than the figure on the 7th day of our experiment. This was easily determined by means of a simple mathematical method and gave us a figure of 473 mg per 100 ml of red blood corpuscles.

We calculated the correlation coefficient between the reticulocyte number and the glutathione content in both groups of animals. This coefficient was highly significant ( $p < 0.001$ ); from this we can infer that the glutathione, abundantly contained in the reticulated erythrocyte, diminishes rapidly down to values characteristic of mature erythrocytes, parallel to the disappearance of the granular and filamentous substance.

These results indicate that the high content of reduced glutathione constitutes a characteristic of the reticulocyte, while the still young but no longer reticulated red blood corpuscles (see data for 12th day of our experiment) reveal a glutathione content which does not differ from that found in the mature erythrocyte. These observations agree with those made by other authors (8, 9), according to whom the young red blood corpuscles -- separated according to the method of fractionated ultracentrifugation -- reveal a reduced glutathione content equal to that found in mature erythrocytes.

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